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Discovery of potent, soluble and orally active TRPV1 antagonists. Structure–activity relationships of a series of isoxazoles

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ABSTRACT

Systematic optimisation of a poorly soluble lead series of isoxazole-3-carboxamides was conducted. Substitution of the 4-position with specific polar functionality afforded the requisite balance of potency, solubility and physicochemical properties. Compound **21a** was found to be efficacious in the rat Capsaicin Hargreaves assay following oral administration.

The TRPV1 (transient receptor potential vanilloid 1) receptor is a well-characterised member of the transient receptor potential (TRP) superfamily of ion channels.[1–3] TRPV1 is located both in the periphery and CNS on C and Aδ fibres, the afferents commonly associated with nociception, and its role in the transmission of pain signalling has been extensively discussed.[4,5] In addition to mediating the effects of exogenous capsaicin (the pungent component of chilli peppers) primary afferent TRPV1 receptors are thought to trigger the actions of heat (>43 °C), and protons (pH <6.8) and are modulated by a variety of endogenous lipid mediators including anandamide and bradykinin.[6] Consequently, TRPV1 is believed to act as an integrator of nociceptive responses to both chemical and thermal noxious stimuli.[7]

TRPV1 knockout mice provided evidence that the channel played a key role in pain, with a clear attenuation of thermal hyperalgesia in response to proinflammatory agents.[8] TRPV1 also shows increased expression in pain states in both rat and human.[9] In addition to the treatment of inflammatory pain, evidence suggests that a number of other disorders, including urinary urge incontinence, cough and irritable bowel syndrome, may be treatable through modulation of TRPV1 signalling.[10]

This level of target validation prompted a subsequent flurry of activity within the pharmaceutical industry.[11] This effort has led to the identification of several TRPV1 antagonists that have entered clinical trials as analgesic agents, and in recent times resulted in the emergence of clinical data for a number of TRPV1 antagonists.[12] The outcomes of these trials have been discussed in detail on a number of occasions already,[11,12] however to date no clear evidence of analgesic efficacy has been demonstrated in man. Should these new chemical entities relieve symptoms of chronic pain in man then this class of compounds may offer one of the first novel mechanisms, in many years, for the treatment of pain. However, the sensitivity of TRPV1 to heat has suggested a role in maintenance of body temperature, and clinical trials of at least one TRPV1 antagonist have been stopped because of dose-limiting hyperthermia that occurred at sub-therapeutic doses.[11]

Indeed, we have previously described the discovery of a novel isoxazole-3-carboxamide series of TRPV1 antagonists, which was optimised to a potent, selective and efficacious compound **1**, Figure 1.[13] Unfortunately, **1** suffered from poor solubility a physicochemical attribute that appears almost ubiquitous within the TRPV1 antagonist pharmacophore.

In this communication, we report optimisation of compound **1** and, in particular, our approaches taken to target TRPV1 antagonists with improved solubility, and lower plasma protein binding,

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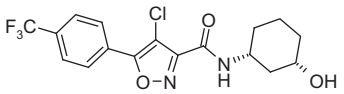


1

Figure 1.

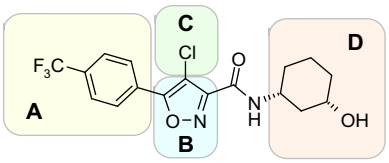


Figure 2.

while maintaining favourable analgesic and pharmacokinetic properties. These efforts culminated in novel ligands with good potency, selectivity and in vivo efficacy following oral administration.

In an effort to introduce solubility, we took three parallel approaches which involved making structural modifications of the molecule as outlined in Figure 2. In particular we expanded on the structure–activity relationships established around the phenyl ring (region A) as, for example, previous work had demonstrated that a meta fluoro substituent could modulate solubility. Secondly variation of the isoxazole ring itself was targeted (region B) and thirdly introduction of polar substituents on the 3-position of the isoxazole ring (region C). The generic routes employed to access these compounds are shown in Schemes 1–3.

The general synthesis of the isoxazole-3-carboxamides has been reported previously,[13] however an alternative approach utilised for this work is outlined in Scheme 1. For example, synthesis of ethyl 5-bromo-4-methylisoxazole-3-carboxylate **3** according to Adembri[14] allowed for a simple Suzuki coupling using the appropriate boronic acid **2**, potassium carbonate and tetrakis(triphenylphosphine)palladium(0) to give ethyl 5-phenyl-4-methylisoxazole-3-carboxylate **4**. Hydrolysis with lithium hydroxide in methanol afforded the acid in near quantitative yield, treatment with oxalyl chloride followed by coupling with the correct amine give the desired amides **7a**–**d** (Table 1).

In an effort to probe the impact of the isoxazole moiety, a number of heterocyclic replacements were synthesized. In the majority of cases this led to an almost complete abolition of TRPV1 antagonist activity (data not shown) although one exception was the reverse isoxazole-5-carboxamides, which could be synthesized according to Scheme 2.

Benzaldehydes **8** were converted to the oxime **9** under standard conditions, stirring with NCS in dimethylformamide and in the presence of HCl in ether gave the chlorooxime **10**. Cyclisation to the isoxazole **11** was achieved by heating the chlorooxime **10**, prop-2-yn-1-ol and triethylamine in toluene. The hydroxyl group was protected as the acetate by performing the halogenation of the isoxazole ring with NCS or NBS in acetic acid. Hydrolysis with LiOH in methanol gave the free alcohol **13** which was oxidised using TEMPO.[15] Amide coupling was performed under standard conditions to afford the desired compounds **16a**–**c** (Table 2).

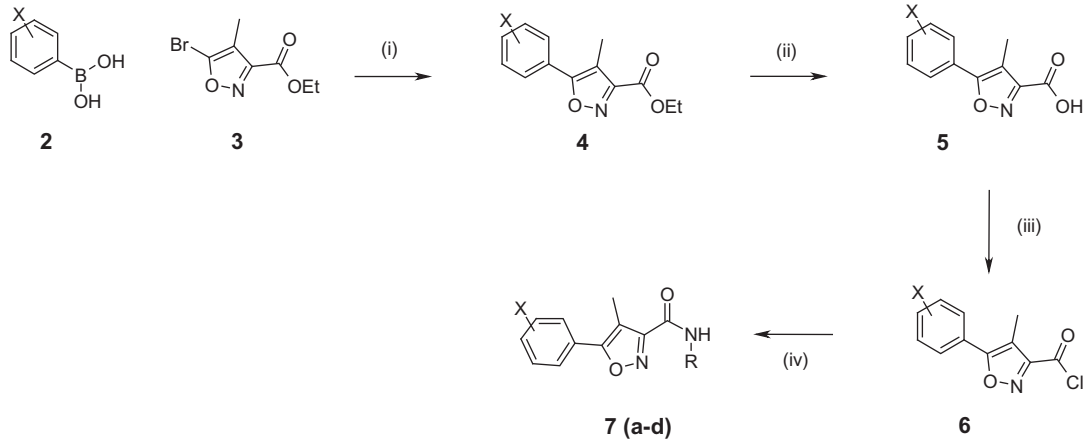
Finally, given the limited investigation around the isoxazole 4-position to date, the influence of further elaboration on solubility, physicochemical parameters and TRPV1 potency was probed. To this end, a range of compounds bearing polar substituents were synthesized according to Schemes 3–5.

Bromination of **4a** was performed with *N*-bromosuccinamide in the presence of benzoyl peroxide, this was then reacted with isopropylamine and sodium bicarbonate to yield the amine **20**. This ester was hydrolysed with lithium hydroxide and the resulting acid coupled with the relevant amine and HATU to afford the desired amides **21a**–**d**. Hydrolysis of the ester **17** with aqueous TFA gave a mixture of the hydroxy acid **18a** and hydroxy ester **18b** which were readily separated. Compounds **19a–i** were synthesised in one of two ways, where R = H, by simply converting the acid **18a** to the amide using 1-propanephosphonic acid cyclic anhdyride (T3P) and the appropriate amine or alternatively where R = Et by reaction of ester **18b** with the appropriate amines in acetonitrile in the presence of triethylamine (Table 3).

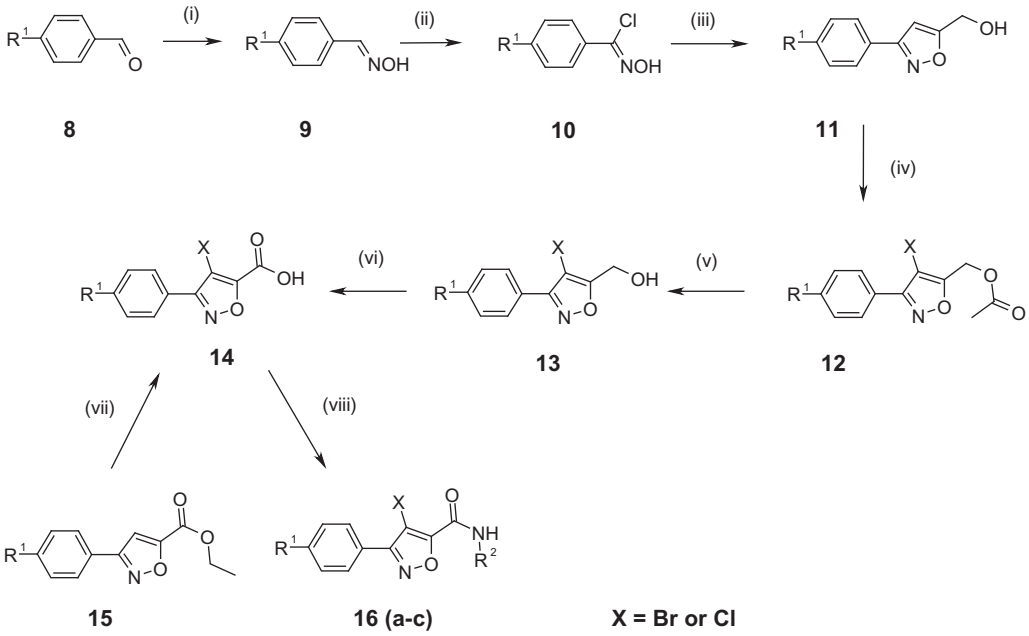
4-Bromo-*N*-cyclopentyl-5-(4-(trifluoromethyl)phenyl)isoxazole-3-carboxamide **22** has been reported previously.[13] This was treated with *n*-butyllithium, acetaldehyde or acetone to yield the respective substituted hydroxylmethyl derivative **23**.

Di-ester **24** was synthesized according to the method of Zhao et al.[16] Selective ^{t}butyl ester hydrolysis under acid conditions with TFA in dichloromethane gave the mono acid **25** in quantitative yield. Subsequent amide coupling was carried out on the ethyl ester with cyclopentylamine in acetonitrile to furnish **26**. This then allowed for treatment of the remaining carboxylic acid with the relevant amine in the presence of T3P® and triethylamine to yield the desired compounds **27a**–**b**.

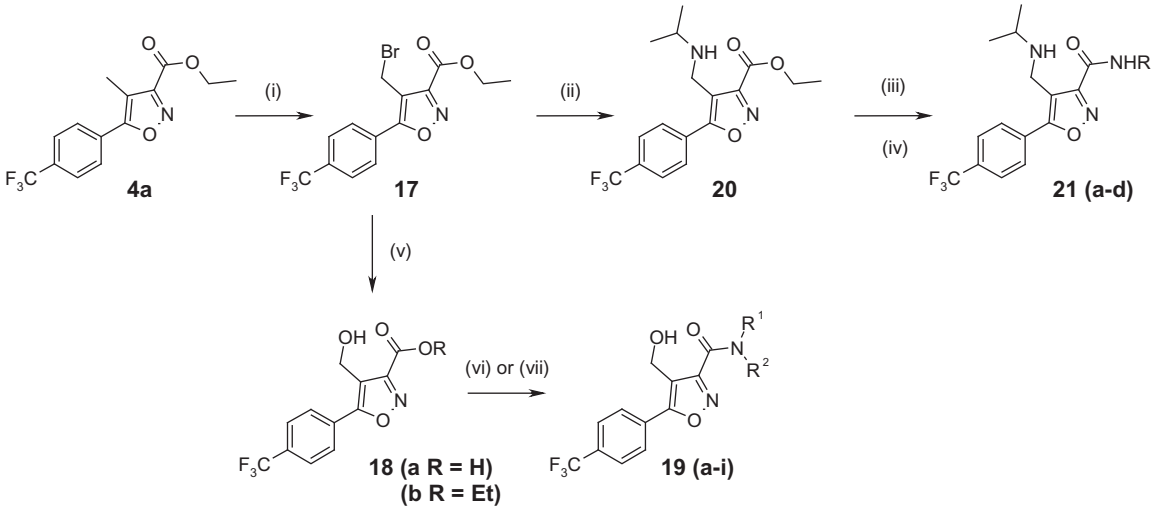
Compounds were evaluated for their ability to modulate influx of Ca^{2+} in Chinese Hamster ovary (CHO-k1) cells stably expressing



Scheme 1. Reagents and conditions: (i) K_2CO_3, tetrakis(triphenylphosphine)palladium(0), aqueous DME, 100 °C; (ii) 1 M lithium hydroxide solution in methanol, aqueous THF, 60 °C; (iii) oxalyl chloride, DCM, DMF, 25 °C; (iv) RNH_2, Et_3N, 25 °C.



Scheme 2. Reagents and conditions: (i) $NH_2OH{\cdot}HCl$, aqueous EtOH, KOH, 25 °C; (ii) NCS, DMF, 25 °C; (iii) prop-2-yn-1-ol, MePh, Et_3N, 25 °C; (iv) AcOH, concd H_2SO_4, NBS or NCS, 120 °C; (v) 1 M lithium hydroxide in methanol, aqueous THF, 60 °C; (vi) TEMPO, NaH_2PO_4, $NaClO_2$, bleach, MeCN, H_2O, 35 °C, then Na_2SO_3, 25 °C; (vii) 1 M lithium hydroxide in methanol, aqueous THF, 60 °C; (viii) HOBt, EDCI, R^2NH_2, Et_3N, DCM, 25 °C.



Scheme 3. Reagents and conditions: (i) NBS, benzoyl peroxide, CCl_4; (ii) isopropylamine, sodium bicarbonate, THF (iii) lithium hydroxide, THF; (iv) NH_2R, HATU, NEt_3, DCM; (v) TFA, H_2O (vi) R^1NH_2, T3P®, NEt_3, DCM; (vii) R^2NH_2, MeCN, NEt_3.

human TRPV1 (VR1) using a Molecular Devices FLIPR. TRPV1 agonists stimulated intracellular fluorescence when applied to the channel, while antagonists inhibited the fluorescent response when co-applied with an agonist. Thus the same TRPV1 transfected cell line was used in both agonist and antagonist assays. Results are reported as pIC_{50} and are an average of at least two independent experiments in duplicate.

Initial investigations centered on incorporation of the 3-fluoro moiety into analogue **1a** to give compound **1b**, however in this instance it led to no beneficial effect on solubility. A similar outcome was achieved for compound **1d**, although in this case a 10-fold increase in potency was observed compared to the des-Fluoro analogue **1c**. Our next attempt to improve intrinsic solubility was to affect a twist around the phenyl-isoxazole bond by the introduction of an *ortho* substituent. Given the scale-able preparation of the 4-methyl isoxazole core, and ability to functionalize the 3- and 5-positions with the requisite groups, a small array of compounds were swiftly prepared according to Scheme 1.Introduction of the *ortho* ethoxy group did indeed have a positive effect on the intrinsic solubility with compounds **7b** and **7c** achieving aqueous solubilities of 27 and 28 mg L^{-1} respectively.[17] However, it resulted in a lowering of potency and the presence of the *ortho* substituent alone was not sufficient to maintain this improvement instead; a combination of the *ortho* substituent and a pendant hydroxyl group in region D was also required for solubility as demonstrated by compound **7d**.

Reversing the substitution on the central isoxazole did result in more soluble compounds as exemplified by **16a**–**c**. However, in general they were less potent at the TRPV1 receptor, with the best compromise coming in the form of **16b**.

Table 1
Structure–activity relationships (SAR) of phenyl and isoxazole rings

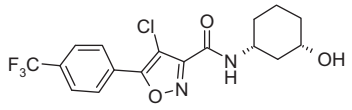
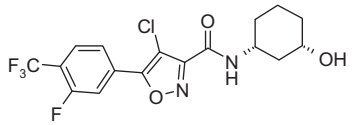
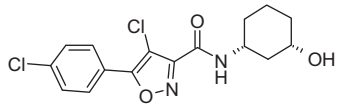
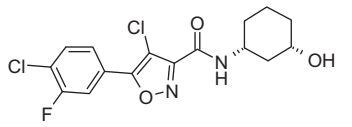
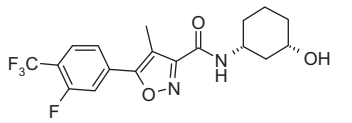
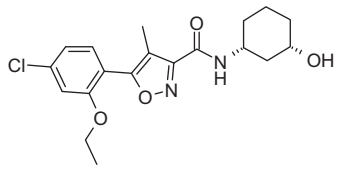
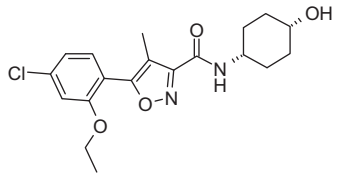
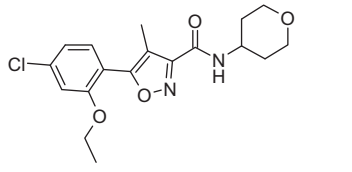
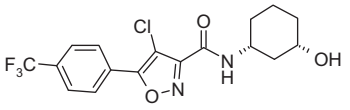
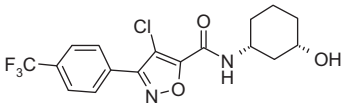
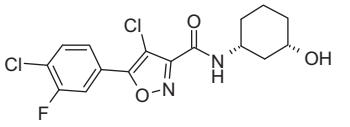
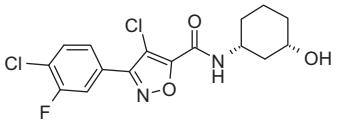
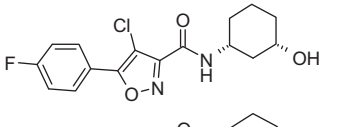
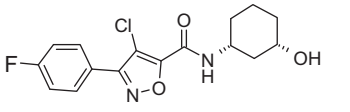
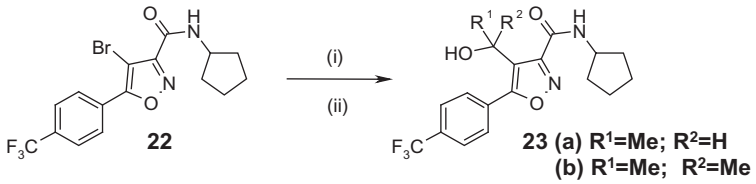
Compound	Structure	TRPV1 pIC_{50}	Solkin (mg L^{-1})[17]
1a		9.3	<1
1b		9.2	<1
1c		8.3	3
1d		9.2	2
7a		8.7	4
7b		7.9	27
7c		6.7	28
7d		7.9	<1

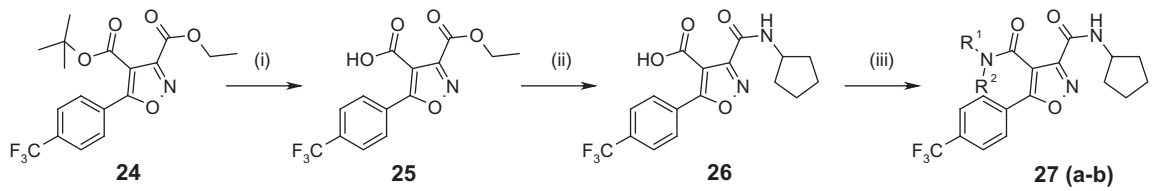
Table 2
Effects of reversing isoxazole ring on potency and solubility

Compound	Structure	TRPV1 pIC_{50}	Solkin (mg L^{-1})[17]
1a		9.3	<1
16a		8.4	5
1d		9.2	2
16b		8.5	11
1e		7.3	38
16c		6.1	47

Finally we explored the SAR around the 4-position of the isoxazole. Introduction of a methyl hydroxyl substituent, in most cases, led to a significant increase in solubility but again a substantial reduction in potency as exemplified by compounds **19a**–**f**. Furthermore, substitution on the methylene adjacent to the isoxazole diminished activity at the TRPV1 receptor (**23a**–**b**). Despite this, compound **19a** showed a good level of in vitro stability, low hERG activity and pleasingly lower plasma protein binding in both human and rat. Encouraged by the tolerance for a polar substituent at the isoxazole 4-position, we embarked upon further exploration of extended polar functionality including acids, amides and amines. This led to compounds **21a**–**b**, **26** and **27a**–**b**. Amides and acids were only weakly tolerated, however introduction of



Scheme 4. Reagents and conditions: (i) ^{n}BuLi, acetaldehyde, THF; (ii) ^{n}BuLi, acetone, THF.



Scheme 5. Reagents and conditions: (i) TFA/DCM; (ii) cyclopentylamine, MeCN; (iii) R^1R^2NH, T3P®, NEt_3, DCM.

Table 3
Effects of water solubilising groups on potency and solubility

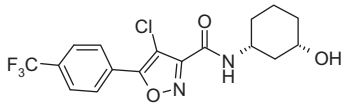
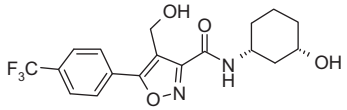
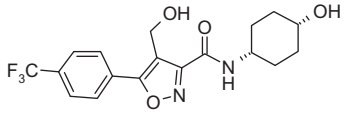
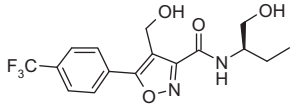
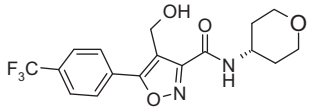
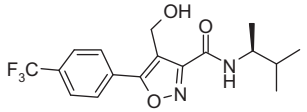
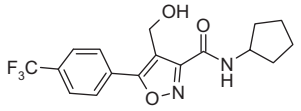
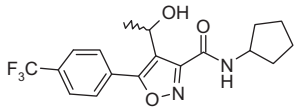
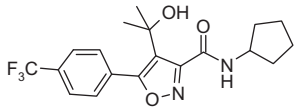
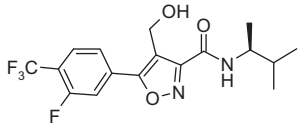
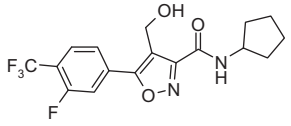
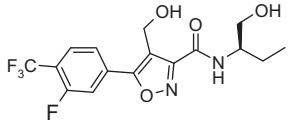
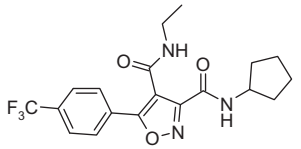
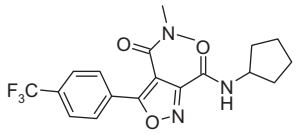
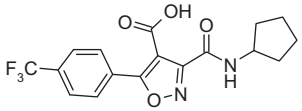
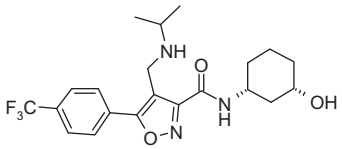
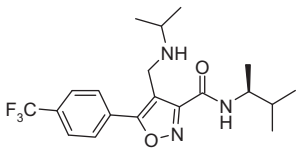
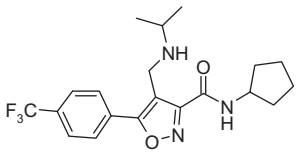
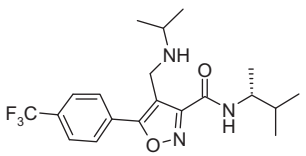
Compound	Structure	TRPV1 pIC_{50}	Solkin (mg L^{-1})[17]
1		9.3	<1
19a		7.1	73
19b		5.8	70
19c		5.8	66
19d		5.5	35
19e		6.9	1.5
19f		6.8	1
23a		6	<1
23b		5.3	<1
19g		7.7	1.4
19h		7.4	73
19i		6.8	31
27a		5.3	1.6
27b		5.7	<1

Table 3 (*continued*)

Compound	Structure	TRPV1 pIC_{50}	Solkin (mg L^{-1})[17]
26		5.8	72
21a		7.2	82
21b		7.2	4
21c		6.5	13
21d		6.1	12

an isopropyl amine in this region achieved compounds such as **21a**, with potency and solubility equivalent to **19a**.

The in vitro profile of compounds **19a** and **21a** are summarised below. As demonstrated in Table 4, both compounds are stable to rat and human hepatic microsomes in vitro. They have lower to moderate affinity for the hERG receptor and moderate to high plasma protein binding.

Compound **21a** was selected for further profiling, and was found to have good stability in human and rat hepatocytes with an intrinsic clearance of <6 μl/min/10^6 cells in both, moderate affinity for hERG with a pK_i of 5.2 and moderate plasma protein binding in both rat and human. As demonstrated in Table 5, compound **21a** has low to moderate clearance in rat, and achieves a relatively long plasma half-life of 7.9 h, perhaps as a consequence of the extensive volume of distribution.

Overall, compound **21a** demonstrated an acceptable pharmacokinetic profile to proceed to in vivo testing. The efficacy of compound **21a** was evaluated in the Capsaicin Hargreaves assay, a functional in vivo assay of TRPV1 induced thermal hyperalgesia in rats.[19] In the Capsaicin Hargreaves model, **21a** (3, 10, 30 μmol/kg; po; 2 h pre-treatment) significantly reversed the thermal hyperalgesia induced by capsaicin, with an MED of 30 μmol/kg (see Fig. 3).

In summary, a series of isoxazole derivatives was optimised through a systematic SAR study of the aryl portion, central isoxazole ring and isoxazole 4-position. This led to the identification of compounds with a better balance of solubility, physicochemical properties and in vitro potency. In particular compounds **19a** and **21a** were identified as the most promising examples from this series and compound **21a** was progressed into animal studies. Despite the lower in vitro potency as compared to compound **1a**, compound **21a** showed clear attenuation of the acute inflammatory thermal response in the rat Capsaicin Hargreaves assay.

Table 4
In vitro pharmacokinetic data for selected compounds

Compound	Solkin ($mg\ L^{-1}$)[17]	HLM CL_{int} ($\mu l\ min^{-1}\ mg^{-1}$)	RLM CL_{int} ($\mu l\ min^{-1}\ mg^{-1}$)	hERG pK_i (% inhibition at 100 μM)	PPB %bound (Human)	PPB %bound (Rat Wistar)
1	<1	<12/<12	<12/<12	Inactive[a]	99.0	99.3
19a	73	<12/27	<12/27	(71%)	91.9	90.9
21a	82	<12/<12	<12/<12	5.2 (81%)	75.0	72.9

Abbreviations: HLM CL_{int}: intrinsic clearance in human liver microsomes; RLM CL_{int}: intrinsic clearance in rat liver microsomes; hERG pK_i: inhibition constant in a hERG channel binding assay (dofetilide); PPB %bound: percentage of compound bound to plasma protein.[18]
[a] Apparent inactivity may have been a result of low solubility under the assay conditions.

Table 5
In vivo pharmacokinetic data for compound **21a**

Compound	CL ($ml\ min^{-1}\ kg^{-1}$)	V_{ss} ($L\ kg^{-1}$)	$T_{1/2}$ (h)	po			%*F*
				C_{max} (μM)	T_{max} (h)	AUC_{last} ($h\ \mu g\ L^{-1}$)	
21a	12.5	5.9	7.9	0.72	2	2728	20

Abbreviations: CL: plasma clearance; V_{ss}: steady state volume of distribution; $T_{1/2}$: biological half-life; C_{max}: peak plasma concentration after oral administration; AUC_{last}: area under the curve at final tested concentration; %*F*: oral bioavailability.

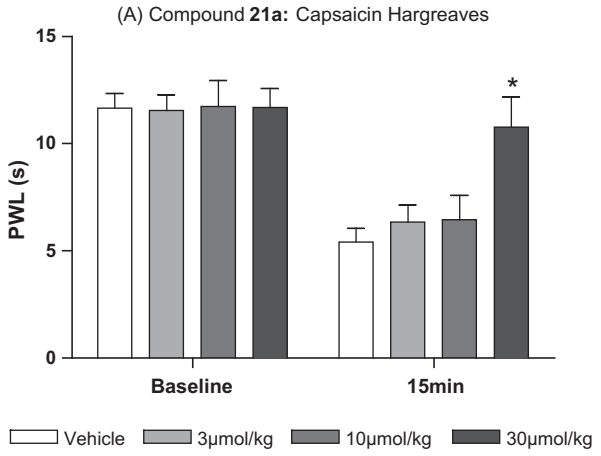


Figure 3. Reversal of induced hyperalgesia by **21a** in the Capsaicin Hargreaves model. Paw withdrawal latency (PWL) is shown on the *y*-axis.

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